flows through the present epidemic area of the Department of Beni.

Summary. Five serologically similar virus strains were recovered in San Joaquin, Bolivia, in 1963 from humans suffering from a newly recognized febrile disease with hemorrhagic manifestations. Three isolates were from splenic tissue obtained at autopsy and 2 from blood specimens taken from acutely ill patients. The prototype strain (Carvallo) was pathogenic for newborn mice and hamsters, produced cytopathic effects in WI-26 cell cultures and plaques in MA-111 cell cultures, was inactivated by chloroform, and was estimated to be 180 mμ or less in size. It was nearly indistinguishable from Junin and Tacaribe viruses by complement fixation test, but completely distinct from these agents by neutralization test. It is proposed that these strains collectively be named Machupo Virus.

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Antibodies in Human Coccidioidomycosis: Immunoelectrophoretic Properties.* (29773)

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In clinically apparent coccidioidomycosis in humans there is a consistent appearance of precipitins and complement-fixing (CF) antibodies. While precipitins have only been useful diagnostically, the titer of CF antibodies, proportional to the severity of the infection, is also of value prognostically(1,2).

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The appearance of these two antibodies follows a regular temporal sequence. Precipitins appear early, being detectable in 90% of patients with primary nondisseminating coccidioidomycosis by the third week of illness. By that time less than one-third of patients have produced CF antibodies. In later weeks of illness, the occurrence of precipitins diminishes, whereas the sera of an increasing proportion of patients contain CF antibodies. By the fifth month of illness, 85% show CF antibodies, but fewer than 25% have demonstrable precipitins.

Examples of variation in antibody type with prolonged antigenic stimulation are known in other clinical immunologic systems, *e.g.*, "saline-" followed by "albumin-" Rh agglutinins (which more readily traverse the placenta) in the materno-fetal interaction (3), and in brucellosis agglutinins followed by "blocking antibodies"(4). In both instances the early antibody is reportedly of higher molecular weight than the later antibody(5,6). Experimental induction of early heavy antibodies and late lighter antibodies has been reported in man by LoSpalluto *et al* (7). We speculated that the early coccidioidal precipitins might constitute larger globulin species than later CF antibodies (which are known to pass the placenta(1,2)), and undertook to compare them using immunodiffusion and immunoelectrophoresis.

Early efforts in immunodiffusion using coccidioidal antigens and human antisera gave inconsistent results(8). However, Huppert(9), using autolysates of *Coccidioides immitis* has demonstrated immunodiffusion lines with regularity in human sera. These lines resulted consistently only with sera containing CF antibodies, although in some instances precipitin-containing sera gave lines of reaction. Rowe *et al*(10) have also demonstrated immunodiffusion lines with human sera from cases of coccidioidomycosis. In addition, they obtained a "gamma globulin" line in immunoelectrophoresis of serum from a rabbit infected with *C. immitis*.

Materials and methods. Human sera containing either or both precipitin and CF antibodies were used in the present studies. Several cerebrospinal fluid specimens from patients with coccidioidal meningitis, showing CF activity, were also included. Most of these specimens had been stored frozen at —15°C from several days to months.

We have found that immunodiffusion lines can be demonstrated regularly in precipitin-containing sera concentrated 5- to 10-fold by dialysis against polyethylene glycol (Carbowax 20,000) at room temperature.‡

Antigens were prepared from young mycelia of *C. immitis* strain Silveira by autolysis at 37°C in the presence of toluene(11). In some instances it was found that dilution of the antigen solution was necessary to provide optimal proportions of antigen and antibody in the demonstration of lines with precipitin sera possibly similar to prezoning inhibition in the tube precipitin test by excess antigen(1). Most immunoelectrophoresis patterns were obtained with one lot of antigen (T Silveira 58), diluted 1:5 to demonstrate CF serum reactants, and diluted 1:50 to demonstrate immunodiffusion lines with precipitin sera.

Immunoelectrophoresis was conducted essentially as described by Grabar(12) or by the microslide modification of Scheidegger (13). Globulins separated by electrophoresis of test sera were demonstrated by goat antisera prepared against specific human serum globulin fractions.§

Results and conclusions. Lines produced in immunodiffusion were distinct and well defined with CF sera. With precipitin sera (concentrated) the lines were distinct but more diffuse in appearance and were demonstrated better with more dilute antigen than used with CF sera. Heating at 56°C for 30 minues to inactivate complement did not prevent the formation of immunodiffusion lines.

A composite of immunoelectrophoretic characteristics is shown in Fig. 1. Immunoelectrophoresis of CF sera (wells E, F, H) gave a reaction line (with T Silveira 58 antigen 1:5 in the lateral troughs 6 and 9) which was distinct and well defined as in immunodiffusion. We were unable to demonstrate immunoelectrophoresis lines with CF sera of

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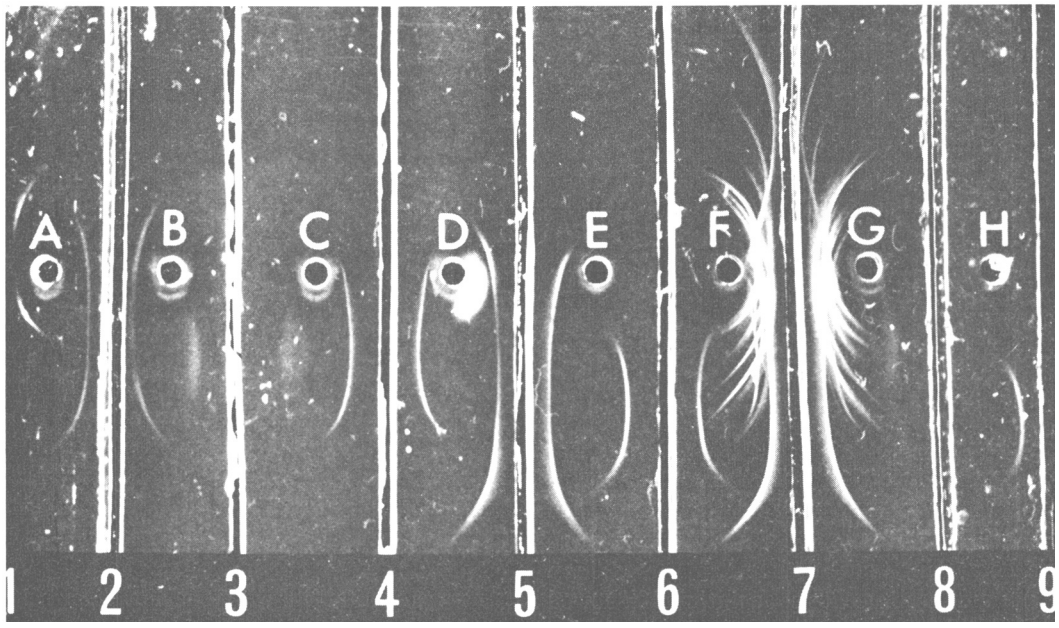

FIG. 1. Immunoelectrophoresis of human sera with 2 types of coccidioidal antibodies. Wells: A, E, F, H contained complement-fixing sera (no precipitins); B, C, D, G contained precipitin sera (no CF antibodies). Troughs: 1 contained goat anti-human alpha$_2$ macroglobulin; 2, goat anti-human beta$_{2A}$ globulin; 3, coccidioidal antigen diluted 1:50; 4, anti-human beta$_2$ macroglobulin; 5, anti-human 7S gamma globulin; 6, coccidioidal antigen 1:5; 7, anti-whole human serum; 8, coccidioidal antigen 1:50; 9, coccidioidal antigen 1:5.

low titer, *e.g.*, up to 1:8; however, when these low titer sera were concentrated against Carbowax 20,000, precipitation lines appeared similar to those of high titer sera. The reaction line obtained after immunoelectrophoresis of concentrated precipitin sera (wells B, C and G) yielded a distinct but more diffuse line similar to that observed in immunodiffusion. Demonstration of the line with precipitin sera (without CF antibodies) was critically dependent on use of a more dilute antigen than that required with CF sera. In the case of the preparation T Silveira 58, a 1:50 dilution appeared roughly optimal—a 10-fold dilution of that needed for good immunodiffusion lines with CF sera. All lines obtained in immunoelectrophoresis with precipitin sera and with CF sera were located in the gamma globulin range with the former always nearer the origin. This was confirmed by mixing CF-negative, precipitin-positive serum with CF-positive, precipitin-negative serum, and with precipitin-positive, CF-positive sera from individual patients. In an effort to establish the position of the lines more precisely, immunoelectrophoresis was conducted with CF serum in a central well flanked by two troughs. One trough was filled with goat anti-human 7S gamma globulin; the twin trough was filled with T Silveira 58 1:5 antigen solution. The arc produced by reaction of the serum with the anti-7S globulin extended over most of the length of the agar on the cathode side (wells D and E, trough 5). The arc produced by the CF serum with coccidioides antigen was roughly at the position of the middle two quarters of the length of the anti-7S line (wells E, F and H, troughs 6 and 9). Arcs of the same form and position were produced by the cerebrospinal fluid specimens with CF activity.

Similarly, immunoelectrophoresis of precipitin serum was conducted with goat anti-19S macroglobulin and T Silveira 58 1:50 antigen in the adjacent troughs. The arc produced by interaction of the serum and anti-19S macroglobulin was roughly half the length of that produced by the anti-7S globulin, but also extended from the origin toward

the cathode (wells C and D, trough 4). The line produced by the 1:50 dilution of antigen and electrophoresed serum lay at the position of the middle two quarters of the anti-19S macroglobulin arc (wells B, C and G, troughs 3 and 8). It was thus considerably nearer the origin than the line produced by CF serum and 1:5 antigen. This line was not observed with concentrated normal sera nor with concentrated sera containing only CF antibodies.

These results have been obtained with precipitin sera from 12 patients, and with CF sera from 16 patients.

In order to aid location of the arcs produced by CF sera, and by precipitin sera, goat antisera against whole human serum and against β_{2A} globulin and α_2 macroglobulin were applied to the longitudinal troughs as above. The α_2 macroglobulin yielded an arc predominantly on the anode side of the serum well. The β_{2A} globulin yielded a line overlapping with the longitudinal positions of the 19S globulin arc and the arc produced by precipitin serum (wells A and B, trough 2); however, it extended considerably beyond the central well toward the anode, and was located closer to the longitudinal trough than either the 19S macroglobulin line or the arc of the precipitin serum. Immunoelectrophoresis of serum from a patient who produced precipitins and CF antibodies concomitantly is depicted in Fig. 2. Two distinct lines of differing mobility are evident.

We have tentatively concluded that in human coccidioidal infections, the precipitins produced are included in 19S β_2 (γ_1) macroglobulin. Complement fixing sera (and cerebrospinal fluid) contain antibodies which yield precipitate with coccidioidal antigen in agar diffusion and they appear from immunoelectrophoresis to have the mobility of 7S γ_2 globulin. It must be emphasized, however, that the assignment of 19S or 7S designations to precipitins demonstrated by immunoelectrophoresis is presumptive only. For example, from the present findings it cannot be excluded that the lines produced by precipitin sera are not also due to 7S globulins, elicited by cross reaction with the anti-19S β_2 macroglobulin preparation. It will be necessary to obtain ultra-centrifugal sedimentation characteristics of the immunoelectrophoretic reactants. It is of interest that lines of precipitation can be demonstrated with CF sera which have not given precipitin reactions in the test tube. Attempts to induce a precipitin reaction in the test tube by altering the physicochemical environment, *e.g.*, in 15% human albumin or in 5% sodium chloride(14) were unsuccessful. The specific relationship between the precipitin activity of CF sera in agar and the CF activity in the test tube remains to be clarified.

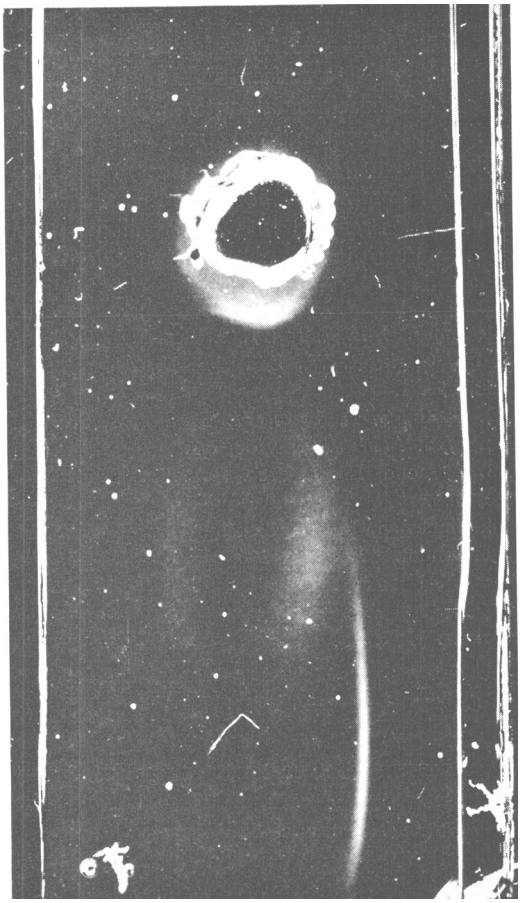

FIG. 2. Immunoelectrophoresis of human serum (concentrated) containing *both* precipitin and complement-fixing antibodies showing distinct lines of precipitation in agar, the former giving diffuse lines nearer origin, the latter giving single more distinct line. Left trough, coccidioidal antigen 1:50; right, coccidioidal antigen 1:5.

Summary. An immunoelectrophoretic study was undertaken on two types of antibody

detectable in human coccidioidomycosis, early precipitins and later complement-fixing antibodies. By using concentrated precipitin sera and dilute coccidioidal antigen, arcs of precipitation were produced which appeared to correspond to the mobility of 19S β_2 macroglobulin. Complement-fixing sera and cerebrospinal fluids reacted with coccidioidal antigen to give precipitation arcs with the mobility of 7S γ_2 globulin.

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A Premixed Powder for Preparation of Tissue Culture Media.* (29774)

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During the development of a program for certification and preservation of cells sponsored by the Cell Culture Collection Committee(1), cooperating laboratories were frequently unable to confirm results with a given cell line because of variations in batches of media prepared in different laboratories. A partial solution was to exchange prepared media along with cell cultures, a common practice between tissue culture laboratories, but of limited applicability. Another measure adopted was to coordinate orders for 10× and 100× liquid media so each laboratory received a large quantity of the same lot. However, this solution is limited by the lot size of liquid media, stability on storage, and by cold storage space possessed by each laboratory. Similar problems have frequently been encountered in laboratories attempting to grow human diploid cell strains for the first time, and investigation usually indicated that the recommended medium had not been used (2).

One approach to the solution of some of these problems has been development of entirely synthetic media. Although the use of synthetic media for growth and maintenance of several cell cultures has been successful(3, 4), the majority of animal cell cultures utilized in most laboratories still requires a medium supplemented with some type of natural product. In lieu of synthetic media, various schemes have been devised by research investigators and commercial supply houses for standardizing the basic constituents of culture media, *i.e.*, the serum and the organic and inorganic chemicals. All these procedures had some degree of success, but the basic problems still remain.

One recent advance which appears to solve most of the problems is the development of

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